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Full length Research Paper

Nine linked SNPs found in goat *melanophilin* (*mlph*) gene

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Melanophilin (*mlph*) gene was characterized as the candidate gene for dilute coat color in human, mice and dog, but little is known in goat. Nine linked SNPs were found in goat *mlph* gene by sequencing a total of 108 individuals from 5 goat populations. No homozygous mutation of the linked SNPs was detected, so we made a hypothesis that the mutation allele might be or might be linked with a recessive lethal gene. In addition, the nine mutational sites as well as a 205 bp coding region in the sequenced segment were compared with homologous sites or region from other species. Result showed that the overall mean distance (p-distance model) and Std. Err are 0.35 and 0.02 among goat, sheep and other eight mammals for the 205 bp coding region. Phylogenetic analysis showed that the codon used in primates may be more similar to that in bovid rather than in rodent animals.

Key words: Melanophilin, goat, coding SNPs, linkage.

INTRODUCTION

Melanophilin, together with myosin Va and Rab27A in mammalian, was characterized to form a tripartite protein complex, taking responsible for transfering melanosomes from the cell body to the tips of their dendrites by an actin-dependent movement (Matesic et al., 2001). Defects in the transfer process can cause pigment dilution of skin and hair in human diseases (Fukuda et al., 2002; Menasche et al., 2003; Kuroda et al., 2005) and the corresponding coat-color mutant mice (Provance et al., 2002; Fukuda and Kuroda, 2004). Among the three candidate gene (*mlph*, *Rab27a*, and *Myo5a*) for dilute coat color phenotype, mutation in *mlph* gene was responsible for color dilution without any future impair- ment in human Griscelli syndrome 3 (GS3) patients or leaden mice, thus it was considered as the most suitable candidate gene for color dilution (Matesic et al., 2001; Menasche et al., 2003; Philipp et al., 2005).

Genetic effect of dilute coat color associated with *mlph* gene mutation has been reported in mice (Menasche et al., 2003), cats (Ishida et al., 2006), and dogs (Philipp et al., 2005; Philipp et al., 2005; Drogemuller et al., 2007). No paper was published on *mlph* gene for ruminant. In order to extend knowledge of *mlph* gene and provide some useful information for goat breeding, sequence determination and characterization of goat *mlph* gene are being processed. In this paper, we reported 9 linked SNPs in goat *mlph* gene and their variation within and among different species.

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MATERIALS AND METHODS

Goat population used and PCR amplification

A total of 108 goat individuals with detailed coat color records from 6 breeds in China were used. The sample size of each population and their distribution were shown in Figure 1 and Table 1.

Genomic DNA from goat blood sample was isolated according to the standard phenol: chloroform extraction method. According to the *mlph* gene sequence of sheep we have got previously (GeneBank accession number: EU218540), the primers we used for PCR amplification were designed as follows: Forward: 5' TGAAAGGGAGTTGAATTGCT 3', and reverse: 5' CACGGTCAAGCGCACTTAC 3'. The PCR product containing exon 8 was 382 bp, which had been submitted to GeneBank (EU195227). PCR amplification was carried out with a total reaction volume of 50 µL containing 150 ng DNA, 400 pmol/L each forward and reverse

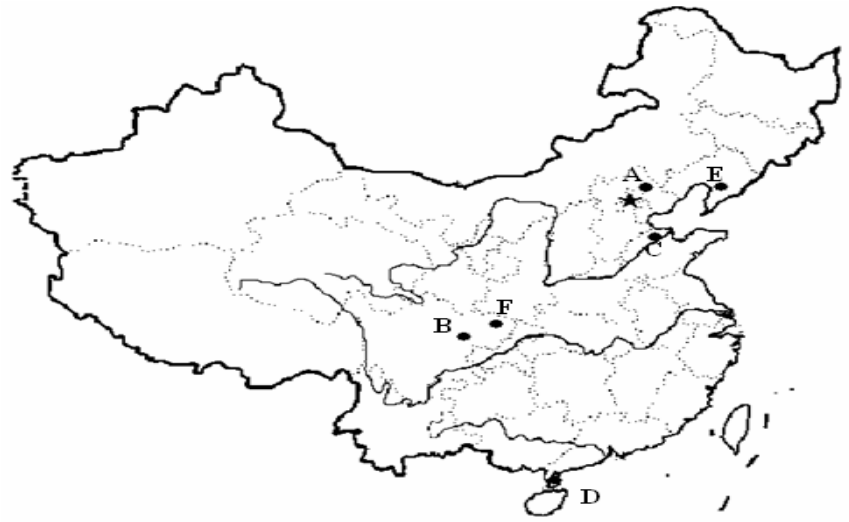

Figure 1. Geographic distribution of six goat populations in China (A: Chengde polled goat; B: Chengdu Ma goat; C: Jining gray goat; D: Leizhou black goat; E: Liaoning cashmere goat; F: Nanjiang brown goat Black strain).

Table 1. Genetype and gene frequency of the linked SNPs in six goat populations.

Goat population	Coat color	Population size	* Individual number of Genetype			Gene frequencies	
			AA(%)	AB(%)	BB(%)	A%	B%
Chengdu Ma goat	Brown (Dilute)	21	21 (100)	0 (0)	0 (0)	100	0
Jining gray goat	**Gray (Dilute)	25	20 (80)	5(20)	0 (0)	90	10
Leizhou black goat	Black	26	16 (61.54)	10 (38.46)	0 (0)	80.77	19.23
Liaoning cashmere goat	White	15	14 (93.33)	1 (6.67)	0 (0)	96.67	3.33
Nanjiang brown goat (Black strain)	Black	21	21 (100)	0 (0)	0 (0)	100	0
In total	-	108	92 (85.19)	16 (14.81)	0 (0)	92.59	7.41

*The percentages in brackets are frequencies comparing to their corresponding population size. ** Not strict, several individuals exhibited mottle or other colors.

primer, 5 µL 10×PCR reaction buffer (Mg^{2+} plus), 200 pmol/µL dNTPs, and 2 U Taq DNA polymerase from TIANGEN BIOTECH Co., Ltd (Beijing, China).

The PCR protocol was as follows: denaturizing at 94°C for 4 min, followed by 35 amplification cycles comprising denaturizing at 94°C for 30 s, annealing at 54°C, and extension at 72°C for 30 s, followed by an extended elongation at 72°C for 10 min. PCR products were detected on 2% agarose gel including 0.5 µg/mL of ethidium bromide, photographed under UV light, and sequenced by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. (Shanghai, China).

Coding region and SNPs identification

First, we retrieved cattle genome sequence containing *mlph* gene from Mapviewer (NW_001494842.1) and the corresponding mRNA sequence (NM_001081597.1). Then the exons were numbered by aligning genome and mRNA sequences using Spidey program (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/). Coding region of goat sequence we determined was concluded by comparing with the catttle protein data (NP_001075066.1), and the SNPs among the 108 individuals were detected by aligning, together with examining the sequencing chromatograms carefully for heterozygote at the SNP sites.

Sequence analysis among species

For comparison and identification of the melanophilin polypeptide product, sequences of other species with a wide zootaxy range were obtained from GenBank (Table 2), together with sheep sequence (EU218540) mentioned above. The coding region identified was aligned with those available for other species, using ClustalW program as implemented in BioEdit (Version 7.0.5.2). Pairwise nucleotide and amino acid sequence divergences were calculated, and the phylogenetic tree among species was reconstructed using the MEGA version 3.1 (Kumar et al., 2004).

Table 2. *MLPH* mRNA and protein sequences of different species from GeneBank.

Species	mRNA Accession No.	Protein Accession No.
Monodelphis domestica	XM_001375200.1	XP_001375237.1
Mus musculus	AF384098.1	NP_443748.2
Rattus norvegicus	NM_001012135.1	AAH81894.1
Homo sapiens	NM_024101.5	AAH01653.1
Pan troglodytes	XM_516180.2	XP_516180.2
Canis familiaris	XM_843654.1	NP_001096689.2
Felis catus	DQ469742.1	NP_001073123.1
Gallus gallus	XM_421876.2	XP_421876.2
Bos taurus	BC133411.1	AAI33412.1
Danio rerio	NM_001079679.1	NP_001073147.1

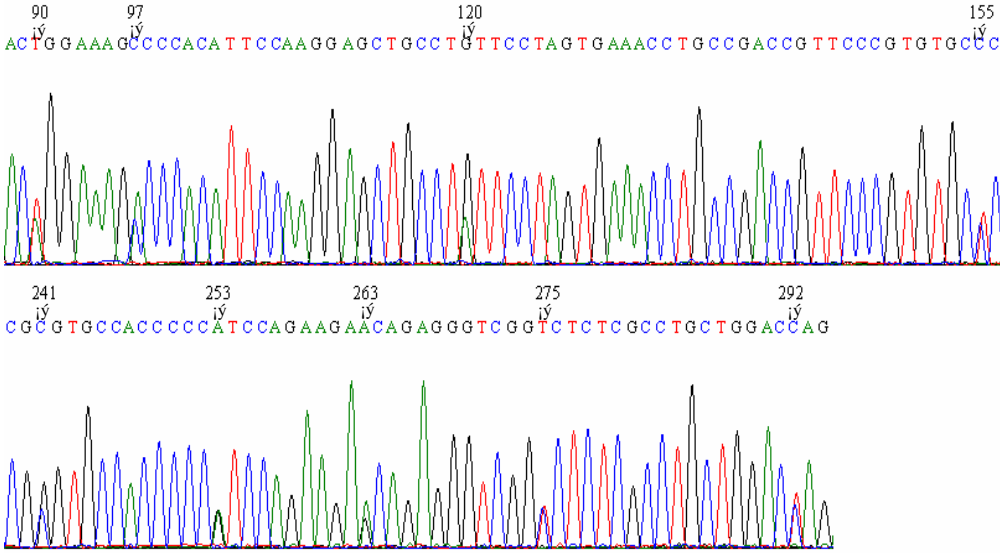

Figure 2. Nine SNPs in heterozygote sequencing chromatograms (Numbers labeled above are base positions of the SNPs in the 382 bp sequence).

RESULTS AND DISCUSSION

Coding region in the sequence determined

The 205 bp coding region was defined by aligning with cattle protein data (AAI33412.1), with the boundaries determined by GT-AG rule. When aligned with the corresponding cattle protein sequence using blastx program, the 205 bp coding region in goat showed high similarity with polypeptide from cattle exon 8 (Identities = 94%, E value = 5e-038), based on which we deduced it to be exon 8 of goat *mlph* gene with 71.22% of GC content.

Completely linked SNPs detected in goat *mlph* gene

Among the 108 determined sequences, nine SNPs in total were detected, 90 (A→T), 97 (C→A), 120 (A→G), 155 (C→T), 241(C→G), 253 (G→A), 263 (G→A), 275 (C→T), and 292 (C→T) respectively, corresponding to the goat *mlph* gene we sent to GeneBank (382bp, EU195227), of which the first four SNPs as stated above were in intron 7 and the rest five were in exon 8.

Among the five SNPs in exon 8, two were synonymous mutation at the position 263 and 275, while three were missense mutation at the position 241(Ala→Gly), 253(Arg→His), and 292(Pro→Leu). An interesting thing was found that only two haplotypes were found, ACACCGGCC represented as A and TAGTGAATT represented as B with frequency of 92.59% and 7.41% respectively (Table 1). Meanwhile, only two genotypes were detected in all individuals, AA and AB as shown in Figure 2, with the frequency of 85.19 and 14.81% respectively. No BB was found. So we deduced that the nine SNPs were completely linked and allele B might be linked with a recessive lethal gene. Given that, mutation in

Table 3. Base information in the five coding SNPs among ruminant available.

Species (haplotype)	Base information of the five SNPs found in goat mlph gene exon 8				
	241	253	263	275	292
Goat (wild type)	C	G	G	C	C
Goat (mutation)	G	A	A	T	T
Sheep	G	G	G	C	T
Cattle	G	G	G	C	C

Table 4. The disparity index test matrix generated from 12 species (1000 reps; seed = 86885).

	1	2	3	4	5	6	7	8	9	10	11	12
1 Goat		0.000	0.000	0.204	0.265	0.020	0.041	0.449	0.306	0.306	0.633	1.388
2 Sheep	1.000		0.000	0.143	0.347	0.082	0.061	0.408	0.224	0.184	0.571	1.224
3 Cattle	1.000	1.000		0.204	0.367	0.000	0.000	0.265	0.245	0.204	0.408	1.102
4 Cat	0.150	0.209	0.126		0.776	0.000	0.000	0.143	0.327	0.000	0.265	0.571
5 Dog	0.141	0.106	0.086	0.008		0.286	0.367	1.224	0.245	0.571	1.490	1.551
6 Opossum	0.417	0.332	1.000	1.000	0.191		0.000	0.388	0.082	0.286	0.000	0.449
7 House mouse	0.407	0.350	1.000	1.000	0.075	1.000		0.143	0.000	0.000	0.102	0.551
8 Norway rat	0.054	0.071	0.121	0.244	0.002	0.150	0.040		0.429	0.000	0.306	0.694
9 Human	0.149	0.194	0.159	0.091	0.166	0.345	1.000	0.054		0.041	0.796	0.918
10 Chimpanzee	0.144	0.244	0.239	1.000	0.040	0.219	1.000	1.000	0.248		0.714	0.837
11 Chicken	0.080	0.091	0.149	0.213	0.003	1.000	0.345	0.174	0.040	0.044		0.000
12 Zebrafish	0.005	0.007	0.007	0.077	0.006	0.093	0.090	0.057	0.030	0.029	1.000	

*The upper triangle are pairwise disparity index, and the lower triangle Probability computed (must be <0.05 for hypothesis rejection at 5% level [gray background]).

mlph gene would cause future impairment at least in goat, and it would be a supplementary to previous study on the relationship between coat color and viability in mammalian (Matesic et al., 2001; Menasche et al., 2003; Philipp et al., 2005). Certainly, the hypothesis of recessive lethal effect of BB needs to be investigated in future.

It was also found that allele B was not found in Chengdu Ma goat and Nanjiang brown goat (Black strain). Nanjiang brown goat was bred with a pedigree of Chengdu Ma goat. So the absence of allele B might be related with the special dilute coat color (tan) of Chengdu Ma goat.

Comparing of the coding region among species

Base information in the five coding SNPs among ruminant were shown in Table 3. Among the five sites, sheep and cattle shared the same base with goat mutant haplotype (at position 241 and 292) whose homozygote was not determined). While at the three sites (position 253, 263 and 275) sheep and cattle shared the same base with goat wild type haplotype. Combined with the synonymous or missense mutation data determined above, base at position 275 may be the most possible site for the hypothesis of the recessive lethal allele.

Generally speaking, high variance of the *mlph* gene exits among species. Comparing with cattle whole protein sequence, the identities were as follows: cat, 64.1%; dog, 64.0%; human, 59.6%; Norway rat, 53.5%; chimpanzee, 55.0%; house mouse, 54.0%; opossum, 28.2%; chicken, 39.4% and zebra fish, 26.7%. Similarity is much lower than other gene such as kappa-casein gene (Mukesh et al., 2006). For the 205 bp coding region determined, variability was found very high: the overall mean distance (p-distance model) and Std. Err are 0.35 and 0.02 among goat, sheep and the homologous region of eight mammals shown in Table 2. When comparing the sequences among a broader range of taxa (mammal, bird, and fish) the overall mean distance is 0.43 (With Std. Err 0.02). When the values above were recalculated using the deduced protein data, the overall mean distance became much higher: 0.57 (With Std. Err 0.03) among 10 mammals and 0.67 (With Std. Err 0.02) among all the 12 species.

The disparity index test matrix (Table 4) was computed using the UPGMA method (bootstrap value with 1000) based on p-distance of the homologous deduced protein data among the 11 species using Mega 3.1 software with gap/missing data complete deletion parameter. Phylogenetic analysis based on nucleotide sequence of the 12 species revealed grouping of goat close to sheep in one

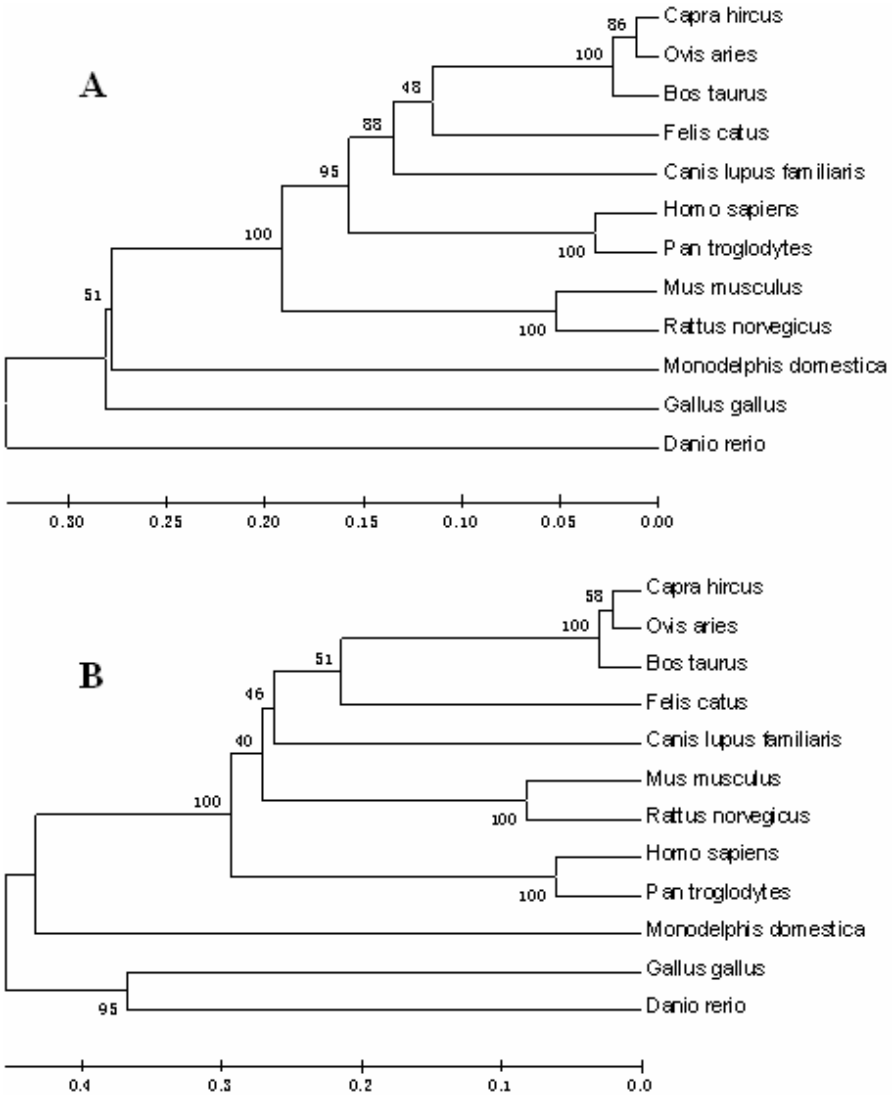

Figure 3. The UPGMA phylogenetic tree based on p-distance of *MLPH* gene (A) exon 8 coding region, and (B) deduced protein sequence data among species.

cluster with cattle forming a separate cluster close to them. However, the primates and rodent formed separate lineages, with rodent at the outer position, and opossum being placed most distantly excluding the non-mammals in the phylogenetic tree (Figure 3 (A)). Based on translated amino acid sequence also, goat, sheep and cattle are grouped close to each other, while primates were placed more distantly than rodent (Figure 3 (B)). The difference between the two phylogenetic tree based on mRNA and protein sequence respectively revealed that the codon used in primates may be nearer with that in bovid animals than in rodent animals.

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